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For: **Gene Encoding Protein Cluster I and the Encoded Protein**

TRANSMITTAL OF CERTIFIED PRIORITY DOCUMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Submitted herewith is a certified copy of Swedish priority application No. 0004325-7 filed November 24, 2000. The priority document is in the English language. It is believed that this satisfies the requirements of 35 U.S.C. §119.

Respectfully submitted,

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PROTEIN CLUSTER I

TECHNICAL FIELD

The present invention relates to the identification of a human gene family expressed in metabolically relevant tissues. The genes encode a group polypeptides referred to as "Protein Cluster I" which are predicted to be useful in the diagnosis of metabolic diseases, such as obesity and diabetes, as well as in the identification of agents useful in the treatment of the said diseases.

BACKGROUND ART

Metabolic diseases are defined as any of the diseases or disorders that disrupt normal metabolism. They may arise from nutritional deficiencies; in connection with diseases of the endocrine system, the liver, or the kidneys; or as a result of genetic defects. Metabolic diseases are conditions caused by an abnormality in one or more of the chemical reactions essential to producing energy, to regenerating cellular constituents, or to eliminating unneeded products arising from these processes. Depending on which metabolic pathway is involved, a single defective chemical reaction may produce consequences that are narrow, involving a single body function, or broad, affecting many organs and systems.

One of the major hormones that influence metabolism is insulin, which is synthesized in the beta cells of the islets of Langerhans of the pancreas. Insulin primarily regulates the direction of metabolism, shifting many processes toward the storage of substrates and away from their degradation. Insulin acts to increase the transport of glucose and amino acids as well as key minerals such as potassium, magnesium, and phosphate from the blood into cells. It also regulates a variety of enzymatic reactions within the cells, all of which have a common overall direction, namely the synthesis of large molecules from small units. A deficiency in the action of insulin (diabetes mellitus) causes severe impairment in (i) the storage of glucose in the form of glycogen and the oxidation of

glucose for energy; (ii) the synthesis and storage of fat from fatty acids and their precursors and the completion of fatty-acid oxidation; and (iii) the synthesis of proteins from amino acids.

There are two varieties of diabetes. Type I is insulin-dependent diabetes mellitus (IDDM), for which insulin injection is required; it was formerly referred to as juvenile onset diabetes. In this type, insulin is not secreted by the pancreas and hence must be taken by injection. Type II, non-insulin-dependent diabetes mellitus (NIDDM) may be controlled by dietary restriction. It derives from insufficient pancreatic insulin secretion and tissue resistance to secreted insulin, which is complicated by subtle changes in the secretion of insulin by the beta cells. Despite their former classifications as juvenile or adult, either type can occur at any age; NIDDM, however, is the most common type, accounting for 90 percent of all diabetes. While the exact causes of diabetes remain obscure, it is evident that NIDDM is linked to heredity and obesity. There is clearly a genetic predisposition to NIDDM diabetes in those who become overweight or obese.

Obesity is usually defined in terms of the body mass index (BMI), i.e. weight (in kilograms) divided by the square of the height (in meters). Weight is regulated with great precision. Regulation of body weight is believed to occur not only in persons of normal weight but also among many obese persons, in whom obesity is attributed to an elevation in the set point around which weight is regulated. The determinants of obesity can be divided into genetic, environmental, and regulatory.

Recent discoveries have helped explain how genes may determine obesity and how they may influence the regulation of body weight. For example, mutations in the *ob* gene have led to massive obesity in mice. Cloning the *ob* gene led to the identification of leptin, a protein coded by this gene; leptin is produced in adipose tissue cells and acts to control body fat. The existence of leptin supports the idea that body weight is regulated, because leptin serves as a signal between adipose tissue and the areas of the brain that control energy metabolism, which influences body weight.

Metabolic diseases like diabetes and obesity are clinically and genetically heterogeneous disorders. Recent advances in molecular genetics have led to the recognition of genes involved in IDDM and in some subtypes of NIDDM, including maturity-onset diabetes of the young (MODY) (Velho & Froguel (1997) Diabetes Metab. 23 Suppl 2:34-37). However, several IDDM susceptibility genes have not yet been identified, and very little is known about genes contributing to common forms of NIDDM. Studies of candidate genes and of genes mapped in animal models of IDDM or NIDDM, as well as whole genome scanning of diabetic families from different populations, should allow the identification of most diabetes susceptibility genes and of the molecular targets for new potential drugs. The identification of genes involved in metabolic disorders will thus contribute to the development of novel predictive and therapeutic approaches.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1

Transmembrane regions identified in the proteins shown as (a) SEQ ID NO: 2; (b) SEQ ID NO: 8; and (c) SEQ ID NO: 6.

DESCRIPTION OF THE INVENTION

According to the present invention, a family of genes and encoded homologous proteins (hereinafter referred to as "Protein Cluster I") has been identified. Consequently, the present invention provides an isolated nucleic acid molecule selected from:

(a) nucleic acid molecules comprising a nucleotide sequence as shown in SEQ ID NO: 1, 3, 5 or 7;

(b) nucleic acid molecules comprising a nucleotide sequence capable of hybridizing, under stringent hybridization conditions, to a nucleotide sequence complementary to the polypeptide coding region of a nucleic acid molecule as defined in (a); and

(c) nucleic acid molecules comprising a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence as defined in (a) or (b).

The nucleic acid molecules according to the present invention includes cDNA, chemically synthesized DNA, DNA isolated by PCR, genomic DNA, and combinations thereof. RNA transcribed from DNA is also encompassed by the present invention.

The term "stringent hybridization conditions" is known in the art from standard protocols (e.g. Ausubel et al., *supra*) and could be understood as e.g. hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at +65°C, and washing in 0.1xSSC / 0.1% SDS at +68°C.

In a preferred form of the invention, the said nucleic acid molecule has a nucleotide sequence identical with SEQ ID NO: 1 of the Sequence Listing. However, the nucleic acid molecule according to the invention is not to be limited strictly to the sequence shown as SEQ ID NO: 1. Rather the invention encompasses nucleic acid molecules carrying modifications like substitutions, small deletions, insertions or inversions, which nevertheless encode proteins having substantially the features of the Protein Cluster I polypeptide according to the invention. Included in the invention are consequently nucleic acid molecules, the nucleotide sequence of which is at least 90% homologous, preferably at least 95% homologous, with the nucleotide sequence shown as SEQ ID NO: 1 in the Sequence Listing.

Included in the invention is also a nucleic acid molecule which nucleotide sequence is degenerate, because of the genetic code, to the nucleotide sequence shown as SEQ ID NO: 1. A sequential grouping of three nucleotides, a "codon", codes for one amino acid. Since there are 64 possible codons, but only 20 natural amino acids, most amino acids are coded for by more than one codon. This natural "degeneracy", or "redundancy", of the genetic code is well known in the art. It will thus be appreciated that the nucleotide sequence shown in the Sequence Listing is only an example within a large but definite group of sequences which will encode the Protein Cluster I polypeptide.

The nucleic acid molecules according to the invention have numerous applications in techniques known to those skilled in the art of molecular biology. These techniques include their use as hybridization probes, for chromosome and gene mapping, in PCR technologies, in the production of sense or antisense nucleic acids, in screening for new therapeutic molecules, etc.

More specifically, the sequence information provided by the invention makes possible large-scale expression of the encoded polypeptides by techniques well known in the art. Nucleic acid molecules of the invention also permit identification and isolation of nucleic acid molecules encoding related polypeptides, such as human allelic variants and species homologues, by well-known techniques including Southern and/or Northern hybridization, and PCR. Knowledge of the sequence of a human DNA also makes possible, through use of Southern hybridization or PCR, the identification of genomic DNA sequences encoding the proteins in Cluster I, expression control regulatory sequences such as promoters, operators, enhancers, repressors, and the like. Nucleic acid molecules of the invention are also useful in hybridization assays to detect the capacity of cells to express the proteins in Cluster I. Nucleic acid molecules of the invention may also provide a basis for diagnostic methods useful for identifying a genetic alteration(s) in a locus that underlies a disease state or states, which information is useful both for diagnosis and for selection of therapeutic strategies.

In a further aspect, the invention provides an isolated polypeptide encoded by the nucleic acid molecule as defined above. In a preferred form, the said polypeptide has an amino acid sequence according to SEQ ID NO: 2, 4, 6 or 8 of the Sequence Listing. However, the polypeptide according to the invention is not to be limited strictly to a polypeptide with an amino acid sequence identical with SEQ ID NO: 2, 4, 6 or 8 in the Sequence Listing. Rather the invention encompasses polypeptides carrying modifications like substitutions, small deletions, insertions or inversions, which polypeptides nevertheless have substantially the features of the Protein Cluster I polypeptide. Included in the invention are consequently polypeptides, the amino acid sequence of which is at least 90% homologous, preferably at least 95% homologous,

with the amino acid sequence shown as SEQ ID NO: 2, 4, 6 or 8 in the Sequence Listing.

In a further aspect, the invention provides a vector harboring the nucleic acid molecule as defined above. The said vector can e.g. be a replicable expression vector, which carries and is capable of mediating the expression of a DNA molecule according to the invention. In the present context the term "replicable" means that the vector is able to replicate in a given type of host cell into which is has been introduced. Examples of vectors are viruses such as bacteriophages, cosmids, plasmids and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known in the art.

Included in the invention is also a cultured host cell harboring a vector according to the invention. Such a host cell can be a prokaryotic cell, a unicellular eukaryotic cell or a cell derived from a multicellular organism. The host cell can thus e.g. be a bacterial cell such as an *E. coli* cell; a cell from a yeast such as *Saccharomyces cervisiae* or *Pichia pastoris*, or a mammalian cell. The methods employed to effect introduction of the vector into the host cell are standard methods well known to a person familiar with recombinant DNA methods.

In yet another aspect, the invention provides a process for production of a polypeptide, comprising culturing a host cell, according to the invention, under conditions whereby said polypeptide is produced, and recovering said polypeptide. The medium used to grow the cells may be any conventional medium suitable for the purpose. A suitable vector may be any of the vectors described above, and an appropriate host cell may be any of the cell types listed above. The methods employed to construct the vector and effect introduction thereof into the host cell may be any methods known for such purposes within the field of recombinant DNA. The recombinant polypeptide expressed by the cells may be secreted, i.e. exported through the cell membrane, dependent on the type of cell and the composition of the vector.

In a further aspect, the invention provides a method for identifying an agent capable of modulating a nucleic acid molecule according to the invention, comprising
(i) providing a cell comprising the said nucleic acid molecule;
(ii) contacting said cell with a candidate agent; and
(iii) monitoring said cell for an effect that is not present in the absence of said candidate agent.

For screening purposes, appropriate host cells can be transformed with a vector having a reporter gene under the control of the nucleic acid molecule according to this invention. The expression of the reporter gene can be measured in the presence or absence of an agent with known activity (i.e. a standard agent) or putative activity (i.e. a "test agent" or "candidate agent"). A change in the level of expression of the reporter gene in the presence of the test agent is compared with that effected by the standard agent. In this way, active agents are identified and their relative potency in this assay determined.

A transfection assay can be a particularly useful screening assay for identifying an effective agent. In a transfection assay, a nucleic acid containing a gene such as a reporter gene that is operably linked to a nucleic acid molecule according to the invention, is transfected into the desired cell type. A test level of reporter gene expression is assayed in the presence of a candidate agent and compared to a control level of expression. An effective agent is identified as an agent that results in a test level of expression that is different than a control level of reporter gene expression, which is the level of expression determined in the absence of the agent. Methods for transfecting cells and a variety of convenient reporter genes are well known in the art (see, for example, Goeddel (ed.), Methods Enzymol., Vol. 185, San Diego: Academic Press, Inc. (1990); see also Sambrook, *supra*).

Throughout this description the terms "standard protocols" and "standard procedures", when used in the context of molecular biology techniques, are to be understood as protocols and procedures found in an ordinary laboratory manual such as: Current Protocols in Molecular Biology, editors F. Ausubel et al., John Wiley and Sons, Inc.

1994, or Sambrook, J., Fritsch, E.F. and Maniatis, T., Molecular Cloning: A laboratory manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1989.

Additional features of the invention will be apparent from the following Examples. Examples 1 to 3 are actual, while Examples 4 to 9 are prophetic.

EXAMPLES

EXAMPLE 1: Identification of protein clusters

A family of homologous proteins (hereinafter referred to as "Protein Cluster I") was identified by an "all-versus-all" BLAST procedure using all *Caenorhabditis elegans* proteins in the Wormpep20 database release (*http://www.sanger.ac.uk/Projects/C_elegans/wormpep/index.shtml*). The Wormpep database contains the predicted proteins from the *C. elegans* genome sequencing project, carried out jointly by the Sanger Centre in Cambridge, UK and the Genome Sequencing Center in St. Louis, USA. A number of 18,940 proteins were retrieved from Wormpep20. The proteins were used in a Smith-Waterman clustering procedure to group together proteins of similarity (Smith T.F. & Waterman M.S. (1981) *Identification of common molecular subsequences.* J. Mol. Biol. 147(1): 195-197; Pearson WR. (1991) *Searching protein sequence libraries: comparison of the sensitivity and selectivity of the Smith-Waterman and FASTA algorithms.* Genomics 11: 635-650; Olsen et al. (1999) *Optimizing Smith-Waterman alignments.* Pac Symp Biocomput.302-313). Completely annotated proteins were filtered out, whereby 10,130 proteins of unknown function could be grouped into 1,800 clusters.

The obtained sequence clusters were compared to the *Drosophila melanogaster* proteins contained in the database Flybase (Berkeley Drosophila Genome Project; *http://www.fruitfly.org*), and annotated clusters were removed. Non-annotated protein clusters, conserved in both *C. elegans* and *D. melanogaster*, were saved to a worm/fly data set, which was used in a BLAST procedure (*http://www.ncbi.nlm.nih.gov/*

Education/BLASTinfo/information3.html) against the Celera Human Genome Database (*http://www.celera.com*). Overlapping fragments were assembled to, as close as possible, full-length proteins using the PHRAP software, developed at the University of Washington *(http://www.genome.washington.edu/ UWGC/analysistools/phrap.htm)*. A group of homologous proteins ("Protein Cluster I") with unknown function was chosen for further studies.

EXAMPLE 2: Analyses of Protein Cluster I

(a) Alignment

The human part of Protein Cluster I comprises polypeptides encoded by three genes (SEQ ID NOS: 1, 5 and 7). In addition, an alternative splicing (corresponding to a deletion of positions 624 to 794 of the gene shown as SEQ ID NO: 1 results in SEQ ID NO: 3. The gene shown as SEQ ID NO: 1 was found to be comprised in a human DNA sequence from clone RP11-108L7 on chromosome 10 (GenBank Accession No. AL133215).

An alignment of the human polypeptides included in Protein Cluster I (SEQ ID NOS: 2, 4, 6 and 8), using the ClustalX multiple alignment software (downloadable from e.g. *ftp://ftp.ebi.ac.uk*) is shown in Table I. For references to the ClustalX software, see Thompson et al. (1997) *The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools*; Nucleic Acids Research, 24:4876-4882. See also Jeanmougin et al. (1998) *Multiple sequence alignment with ClustalX*; Trends Biochem. Sci. 23:403-405. The alignment showed a high degree of conservation in two separate regions, indicating the presence of two novel domains (see positions marked with stars in Table I).

(b) HMM-Pfam

A HMM-Pfam search was performed on the three human family members. Pfam (*http://pfam.wustl.edu*) is a large collection of protein families and domains. Pfam contains multiple protein alignments and profile-HMMs (Profile Hidden Markov Models) of these families. Profile-HMMs can be used to do sensitive database searching using statistical descriptions of a sequence family's consensus. Pfam is available on the WWW at *http://pfam.wustl.edu; http://www.sanger.ac.uk/Software/Pfam*; and *http://www.cgr.ki.se/Pfam*. The latest version (4.3) of Pfam contains 1815 families. These Pfam families match 63% of proteins in SWISS-PROT 37 and TrEMBL 9. For references to Pfam, see Bateman et al. (2000) *The Pfam protein families database*. Nucleic Acids Res. 28:263-266; Sonnhammer et al. (1998) *Pfam: Multiple Sequence Alignments and HMM-Profiles of Protein Domains*. Nucleic Acids Research, 26:322-325; Sonnhammer et al. (1997) *Pfam: a Comprehensive Database of Protein Domain Families Based on Seed Alignments*. Proteins 28:405-420.

The HMM-Pfam search indicated that no previously known domains could be identified in Protein Cluster I.

(c) TM-HMM

The human proteins in Cluster I were analyzed using the TM-HMM tool available at *http://www.cbs.dtu.dk/services/TMHMM-1.0*. TM-HMM is a method to model and predict the location and orientation of alpha helices in membrane-spanning proteins (Sonnhammer et al. (1998) *A hidden Markov model for predicting transmembrane helices in protein sequences*. ISMB 6:175-182). Transmembrane segments were identified in the proteins shown as SEQ ID NOS: 2, 6 and 8 (Fig. 1)

(d) Analysis of non-human orthologs

The *C. elegans* genome includes six genes encoding proteins within Protein Cluster I, of which the closest ancestor in evolution, a sequence included the *C. elegans* cosmid

T04F8.1 (GenBank Accession No. Z66565; see also: *Genome sequence of the nematode C. elegans: a platform for investigating biology;* The *C. elegans* Sequencing Consortium. Science (1998) 282:2012-2018. Published errata appear in Science (1999) 283:35; 283:2103; and 285:1493.) is 53% identical to the three identified human proteins (SEQ ID NOS: 2, 6 and 8).

The *Drosophila melanogaster* genome comprises two genes belonging to Protein Cluster I, of which the closest relative (GenBank Accession No. AE003606_24; see also Adams et al. (2000) *The genome sequence of Drosophila melanogaster*; Science 287:2185-2195) is 53% identical to the human protein set.

The human proteins also show 38% identity to a *Saccharomyces cerevisiae* protein (GenPept Accession No. CAA99495.1). The yeast protein has been annotated in the *Saccharomyces* Genome Database as a putative transporter (*http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=YOR270c*).

Two public *Rattus norvegicus* database entries (GENBANK entries AF276997 and S70011) have been annotated as putative tricarboxylate carrier proteins. The genes have 88% and 79% identity, respectively, with SEQ ID NO: 1. The tricarboxylate carrier transports citrate or other tricarboxylates across the inner membranes of mitochondria in an electroneutral exchange for malate or other dicarboxylic acids. (Azzi et al. (1993) J. Bioenerg. Biomembr. 25: 515-524).

EXAMPLE 3: Expression analysis

EST databases provided by the EMBL (*http://www.embl.org/Services/index.html*) were used to check whether the human proteins in Cluster I were expressed, in order to identify putative pseudogenes. No putative pseudogenes were identified in Protein Cluster I.

The tissue distribution of the human genes was studied using the Incyte LifeSeq® database (*http://www.incyte.com*). The nucleic acid molecule shown as SEQ ID NO: 1 was found to be expressed primarily in the nervous system and the digestive system. The nucleic acid molecule shown as SEQ ID NO: 3 was expressed primarily in male genitalia. The nucleic acid molecule shown as SEQ ID NO: 5 was expressed primarily in the liver and in embryonic structures. The nucleic acid molecule shown as SEQ ID NO: 7 was expressed primarily in the immune system. Therefore, the said nucleic acid molecules shown as SEQ ID NO: 1, 3, 5 and 7 and the polypeptides shown as SEQ ID NO: 2, 4, 6 and 8 are proposed to be useful for differential identification of the tissue(s) or cell types(s) present in a biological sample and for diagnosis of diseases and disorders, including metabolic disorders and immune disorders.

EXAMPLE 4: Multiple Tissue Northern blotting

Multiple Tissue Northern blotting (MTN) is performed to make a more thorough analysis of the expression profiles of the proteins in Cluster I. Multiple Tissue Northern (MTN™) Blots *(http://www.clontech.com/mtn)* are pre-made Northern blots featuring Premium Poly A+ RNA from a variety of different human, mouse, or rat tissues. MTN Blots can be used to analyze size and relative abundance of transcripts in different tissues. MTN Blots can also be used to investigate gene families and alternate splice forms and to assess cross species homology.

EXAMPLE 5: Expressing profiling using microarrays

Microarrays consist of a highly ordered matrix of thousands of different DNA sequences that can be used to measure DNA and RNA variation in applications that include gene expression profiling, comparative genomics and genotyping (For recent reviews, see e.g.: Harrington et al. (2000) *Monitoring gene expression using DNA microarrays*. Curr. Opin. Microbiol. 3(3): 285-291; or Duggan et al. (1999) *Expression profiling using cDNA Microarrays*. Nature Genetics Supplement 21:10-14).

The expression pattern of the proteins in Cluster I can be analyzed using GeneChip® expression arrays (*http://www.affymetrix.com/products/app_exp.html*). Briefly, mRNAs are extracted from various tissues. They are reverse transcribed using a T7-tagged oligo-dT primer and double-stranded cDNAs are generated. These cDNAs are then amplified and labeled using In Vitro Transcription (IVT) with T7 RNA polymerase and biotinylated nucleotides. The populations of cRNAs obtained are purified and fragmented by heat to produce a distribution of RNA fragment sizes from approximately 35 to 200 bases. GeneChip® expression arrays are hybridized with the samples. The arrays are washed and stained. The cartridges are scanned using a confocal scanner and the images are analyzed with the GeneChip 3.1 software (Affymetrix).

EXAMPLE 6: Identification of polypeptides binding to Protein Cluster I

In order to assay for proteins interacting with Protein Cluster I, the two-hybrid screening method can be used. The two-hybrid method, first described by Fields & Song (1989) Nature 340:245-247, is a yeast-based genetic assay to detect protein-protein interactions *in vivo*. The method enables not only identification of interacting proteins, but also results in the immediate availability of the cloned genes for these proteins.

The two-hybrid method can be used to determine if two known proteins (i.e. proteins for which the corresponding genes have been previously cloned) interact. Another important application of the two-hybrid method is to identify previously unknown proteins that interact with a target protein by screening a two-hybrid library. For reviews, see e.g.: Chien et al. (1991) *The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest*. Proc. Natl. Acad. Sci. U.S.A. 88:9578-9582; Bartel PL, Fields (1995) *Analyzing protein-protein interactions using two-hybrid system*. Methods Enzymol. 254:241-263; or Wallach et al. (1998) *The yeast two-hybrid screening technique and its use in the study of protein-protein*

interactions in apoptosis. Curr. Opin. Immunol. 10(2): 131-136. See also *http://www.clontech.com/matchmaker*.

The two-hybrid method uses the restoration of transcriptional activation to indicate the interaction between two proteins. Central to this technique is the fact that many eukaryotic transcriptional activators consist of two physically discrete modular domains: the DNA-binding domain (DNA-BD) that binds to a specific promoter sequence and the activation domain (AD) that directs the RNA polymerase II complex to transcribe the gene downstream of the DNA binding site. The DNA-BD vector is used to generate a fusion of the DNA-BD and a bait protein X, and the AD vector is used to generate a fusion of the AD and another protein Y. An entire library of hybrids with the AD can also be constructed to search for new or unknown proteins that interact with the bait protein. When interaction occurs between the bait protein X and a candidate protein Y, the two functional domains, responsible for DNA binding and activation, are tethered, resulting in functional restoration of transcriptional activation. The two hybrids are cotransformed into a yeast host strain harboring reporter genes containing appropriate upstream binding sites; expression of the reporter genes then indicates interaction between a candidate protein and the target protein.

EXAMPLE 7: Full-length cloning of Cluster I genes

The polymerase chain reaction (PCR), which is a well known procedure for *in vitro* enzymatic amplification of a specific DNA segment, can be used for direct cloning of Protein Cluster I genes. Tissue cDNA can be amplified by PCR and cloned into an appropriate plasmid and sequenced. For reviews, see e.g. Hooft van Huijsduijnen (1998) *PCR-assisted cDNA cloning: a guided tour of the minefield.* Biotechniques 24:390-392; Lenstra (1995) *The applications of the polymerase chain reaction in the life sciences.* Cellular & Molecular Biology 41:603-614; or Rashtchian (1995) *Novel methods for cloning and engineering genes using the polymerase chain reaction.* Current Opinion in Biotechnology 6:30-36. Various methods for generating suitable ends to facilitate the direct cloning of PCR products are given e.g. in Ausubel et al. *supra* (section 15.7).

In an alternative approach to isolate a cDNA clone encoding a full length protein of Protein Cluster I, a DNA fragment corresponding to a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 3, 5 or 7, or a portion thereof, can be used as a probe for hybridization screening of a phage cDNA library. The DNA fragment is amplified by the polymerase chain reaction (PCR) method. The primers are preferably 10 to 25 nucleotides in length and are determined by procedures well known to those skilled in the art. A lambda phage library containing cDNAs cloned into lambda phage-vectors is plated on agar plates with *E. coli* host cells, and grown. Phage plaques are transferred to nylon membranes, which are hybridized with a DNA probe prepared as described above. Positive colonies are isolated from the plates. Plasmids containing cDNA are rescued from the isolated phages by standard methods. Plasmid DNA is isolated from the clones. The size of the insert is determined by digesting the plasmid with appropriate restriction enzymes. The sequence of the entire insert is determined by automated sequencing of the plasmids.

EXAMPLE 8: Recombinant expression of proteins in eukaryotic host cells

To produce proteins of Cluster I, a polypeptide-encoding nucleic acid molecule is expressed in a suitable host cell using a suitable expression vector and standard genetic engineering techniques. For example, the polypeptide-encoding sequence is subcloned into a commercial expression vector and transfected into mammalian, e.g. Chinese Hamster Ovary (CHO), cells using a standard transfection reagent. Cells stably expressing a protein are selected. Optionally, the protein may be purified from the cells using standard chromatographic techniques. To facilitate purification, antisera is raised against one or more synthetic peptide sequences that correspond to portions of the amino acid sequence, and the antisera is used to affinity purify the protein.

EXAMPLE 9: Determination of gene function

Methods are known in the art for elucidating the biological function or mode of action of individual genes. For instance, RNA interference (RNAi) offers a way of specifically and potently inactivating a cloned gene, and is proving a powerful tool for investigating gene function. For reviews, see e.g. Fire (1999) *RNA-triggered gene silencing.* Trends in Genetics 15:358-363; or Kuwabara & Coulson (2000) *RNAi–prospects for a general technique for determining gene function.* Parasitology Today 16:347-349. When double-stranded RNA (dsRNA) corresponding to a sense and antisense sequence of an endogenous mRNA is introduced into a cell, the cognate mRNA is degraded and the gene is silenced. This type of posttranscriptional gene silencing (PTGS) was first discovered in *C. elegans* (Fire et al., (1998) Nature 391:806-811). RNA interference has recently been used for targeting nearly 90% of predicted genes on *C. elegans* chromosome I (Fraser et al. (2000) Nature 408: 325-330) and 96% of predicted genes on *C. elegans* chromosome III (Gönczy et al. (2000) Nature 408:331-336).

TABLE I

Alignment of polypeptides in Protein Cluster I

```
                    *    **  ***** *****  **  ***** * **  **   * **
SEQ ID NO: 2  MESKMGELPLDINIQEPRWDQSTFLGRARHFFTVTDPRNLLLSGAQLEASRNIVQNYRAGVVTPGITEDQLWRAKY (76)
SEQ ID NO: 4  MESKMGELPLDINIQEPRWDQSTFLGRARHFFTVTDPRNLLLSGAQLEASRNIVQNY------------------- (57)
SEQ ID NO: 6  ---MEADLSG-FNIDAPRWDQRTFLGRVKHFLNITDPRTVFVSERELDWAKVMVEKSRMGVVPPGTQVEQLLYAKK (72)
SEQ ID NO: 8  ---MSGELPPNINIKEPRWDQSTFIGRANHFFTVTDPRNILLTNEQLESARKIVHDYRQGIVPPGLTENELWRAKY (73)

                                                *  * ***************
SEQ ID NO: 2  VYDSAFHPDTGEKVVLIGRMSAQVPMNMTITGCMLTFYRKTPTVVFWQWVNQSFNAIVNYSNRSGDTPITVRQLGT (152)
SEQ ID NO: 4  ------------------------------------RKTPTVVFWQWVNQSFNAIV------------------ (77)
SEQ ID NO: 6  LYDSAFHPDTGEKMNVIGRMSFQLPGGMIITGFMLQFYRTMPAVIFWQWVNQSFNALVNYTNRNAASPTSVRQMAL (148)
SEQ ID NO: 8  IYDSAFHPDTGEKMILIGRMSAQVPMNMTITGCMMTFYRTTPAVLFWQWINQSFNAVVNYTNRSGDAPLTVNELGT (149)


SEQ ID NO: 2  AYVSATTGAVATALGLKSLTKHLPPLVGRFVPFAAVAAANCINIPLMRQRELQVGIPVADEAGQRLGYSVTAAKQG (228)
SEQ ID NO: 4  ------------------------------------------------------------------------
SEQ ID NO: 6  SYFTATTTAVATAVGMNMLTKKAPPLVGRWVPFAAVAAANCVNIPMMRQRELIKGICVKDRNENEIGHSRRAAAIG (224)
SEQ ID NO: 8  AYVSATTGAVATALGLNALTKHVSPLIGRFVPFAAVAAANCINIPLMRQRELKVGIPVTDENGNRLGESANAAKQA (225)


SEQ ID NO: 2  IFQVVISRICMAIPAMAIPPLIMDTLEKKDFLK---------------------------------------------- (261)
SEQ ID NO: 4  ----------------------------------------------------------------------------------
SEQ ID NO: 6  ITQVVISRITMSAPGMILLPVIMERLEKLHFMQKVKVLHAPLQVMLSGCFLIFMVPVACGLFPQKCELPVSYLEPKLQDTIKAKYGELEPYVYFNKGL (300)
SEQ ID NO: 8  ITQVVVSRILMAAPGMAIPPFIMNTLEKKAFLKRFPWMSAPIQVGLVGFCLVFATPLCCALFPQKSSMSVTSLEAELQAKIQESHPELR-RVYFNKGL (301)
```

CLAIMS

1. An isolated nucleic acid molecule selected from:
(a) nucleic acid molecules comprising a nucleotide sequence as shown in SEQ ID NO: 1, 3, 5 or 7;
(b) nucleic acid molecules comprising a nucleotide sequence capable of hybridizing, under stringent hybridization conditions, to a nucleotide sequence complementary to the polypeptide coding region of a nucleic acid molecule as defined in (a); and
(c) nucleic acid molecules comprising a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence as defined in (a) or (b).

2. An isolated polypeptide encoded by the nucleic acid molecule according to claim 1.

3. The isolated polypeptide according to claim 2 having an amino acid sequence shown as SEQ ID NO: 2, 4, 6, or 8 in the Sequence Listing

4. A vector harboring the nucleic acid molecule according to claim 1.

5. A replicable expression vector which carries and is capable of mediating the expression of a nucleotide sequence according to claim 1.

6. A cultured host cell harboring a vector according to claim 4 or 5.

7. A process for production of a polypeptide, comprising culturing a host cell according to claim 6 under conditions whereby said polypeptide is produced, and recovering said polypeptide.

8. A method for identifying an agent capable of modulating a nucleic acid molecule according to claim 1, comprising

(i) providing a cell comprising the said nucleic acid molecule;

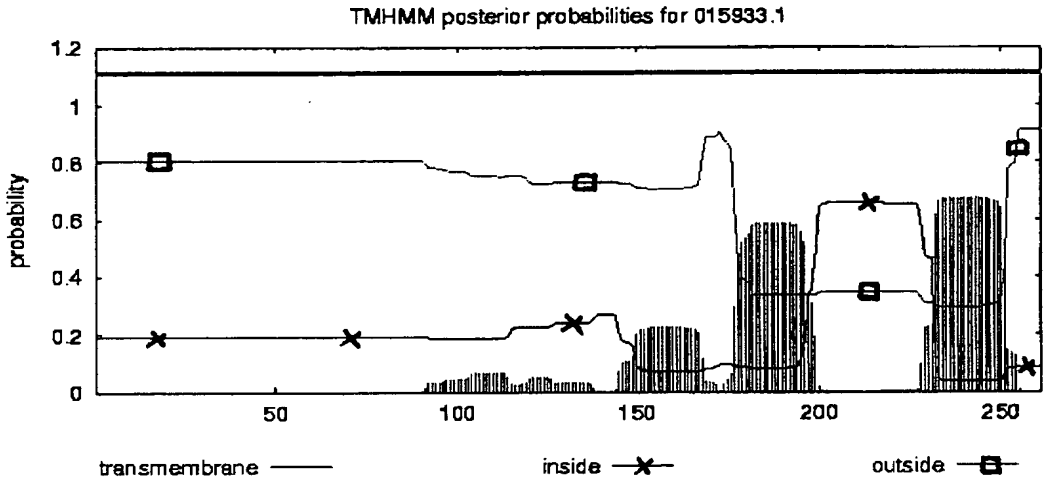
(ii) contacting said cell with a candidate agent; and

(iii) monitoring said cell for an effect that is not present in the absence of said candidate agent.

ABSTRACT

The present invention relates to the identification of a human gene family expressed in metabolically relevant tissues. The genes encode a group polypeptides referred to as "Protein Cluster I" which are predicted to be useful in the diagnosis of metabolic diseases, such as obesity and diabetes, as well as in the identification of agents useful in the treatment of the said diseases.

Fig. 1a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probability
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0.4
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transmembrane
inside
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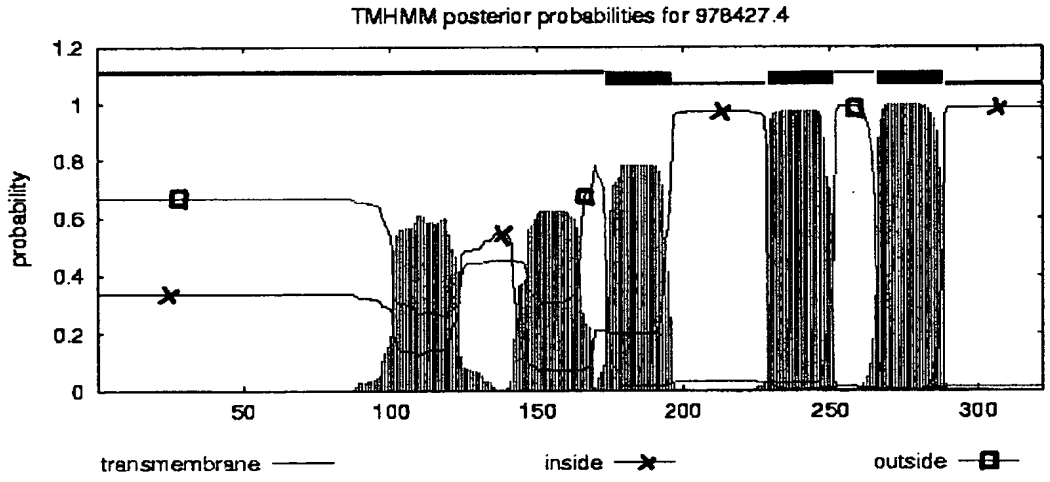
Fig. 1b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inside
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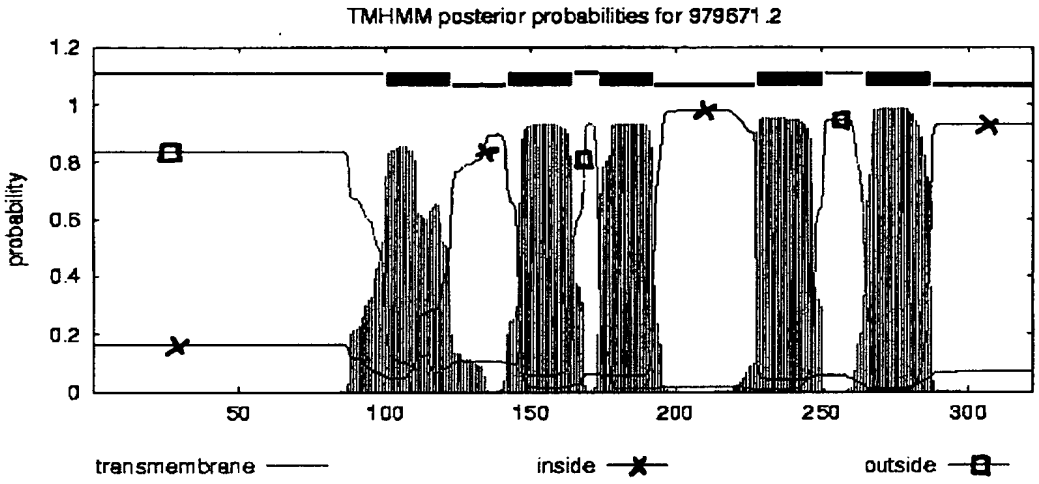
Fig. 1c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transmembrane
inside
outside

SEQUENCE LISTING

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Gly Ile Thr Glu Asp Gln Leu Trp Arg Ala Lys Tyr Val Tyr Asp Ser
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Ala Phe His Pro Asp Thr Gly Glu Lys Val Val Leu Ile Gly Arg Met
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Ser Phe Asn Ala Ile Val Asn Tyr Ser Asn Arg Ser Gly Asp Thr Pro
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Ile Thr Val Arg Gln Leu Gly Thr Ala Tyr Val Ser Ala Thr Thr Gly
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Cys Ile Asn Ile Pro Leu Met Arg Gln Arg Glu Leu Gln Val Gly Ile
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Pro Val Ala Asp Glu Ala Gly Gln Arg Leu Gly Tyr Ser Val Thr Ala
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Ala Lys Gln Gly Ile Phe Gln Val Val Ile Ser Arg Ile Cys Met Ala
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Pro Leu Asp Ile Asn Ile Gln Glu Pro Arg Trp Asp Gln Ser Thr Phe
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25                  30                  35                  40

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gtt ggc ttc tgt ttg gtg ttt gct aca ccc ctg tgt tgt gcc ctg ttt      985
Val Gly Phe Cys Leu Val Phe Ala Thr Pro Leu Cys Cys Ala Leu Phe
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cct cag aaa agt tcc atg tct gtg aca agc ttg gag gcc gag ttg caa     1033
Pro Gln Lys Ser Ser Met Ser Val Thr Ser Leu Glu Ala Glu Leu Gln
        290                 295                 300

gct aag atc caa gag agc cat cct gaa ttg cga cgc gtg tac ttc aat     1081
Ala Lys Ile Gln Glu Ser His Pro Glu Leu Arg Arg Val Tyr Phe Asn
    305                 310                 315

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Lys Gly Leu
320
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tgggctgaat ccagttcatt gttttttttt tggtaagaag tgagactaca gttccagcta 1973

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35 40 45

Lys Ile Val His Asp Tyr Arg Gln Gly Ile Val Pro Pro Gly Leu Thr
50 55 60

Glu Asn Glu Leu Trp Arg Ala Lys Tyr Ile Tyr Asp Ser Ala Phe His
65 70 75 80

Pro Asp Thr Gly Glu Lys Met Ile Leu Ile Gly Arg Met Ser Ala Gln
85 90 95

Val Pro Met Asn Met Thr Ile Thr Gly Cys Met Met Thr Phe Tyr Arg
100 105 110

Thr Thr Pro Ala Val Leu Phe Trp Gln Trp Ile Asn Gln Ser Phe Asn
115 120 125

Ala Val Val Asn Tyr Thr Asn Arg Ser Gly Asp Ala Pro Leu Thr Val
130 135 140

Asn Glu Leu Gly Thr Ala Tyr Val Ser Ala Thr Thr Gly Ala Val Ala
145 150 155 160

Thr Ala Leu Gly Leu Asn Ala Leu Thr Lys His Val Ser Pro Leu Ile
165 170 175

Gly Arg Phe Val Pro Phe Ala Ala Val Ala Ala Ala Asn Cys Ile Asn
180 185 190

Ile Pro Leu Met Arg Gln Arg Glu Leu Lys Val Gly Ile Pro Val Thr
195 200 205

Asp Glu Asn Gly Asn Arg Leu Gly Glu Ser Ala Asn Ala Ala Lys Gln
210 215 220

Ala Ile Thr Gln Val Val Val Ser Arg Ile Leu Met Ala Ala Pro Gly
225 230 235 240

Met Ala Ile Pro Pro Phe Ile Met Asn Thr Leu Glu Lys Lys Ala Phe
245 250 255

Leu Lys Arg Phe Pro Trp Met Ser Ala Pro Ile Gln Val Gly Leu Val
260 265 270

Gly Phe Cys Leu Val Phe Ala Thr Pro Leu Cys Cys Ala Leu Phe Pro
275 280 285

Gln Lys Ser Ser Met Ser Val Thr Ser Leu Glu Ala Glu Leu Gln Ala
290 295 300

Lys Ile Gln Glu Ser His Pro Glu Leu Arg Arg Val Tyr Phe Asn Lys
305 310 315 320

Gly Leu